THE JOURNAL OF INVESTIGATIVE DERMATOLOGY

Vol. 52, No. 3
Printed in U.S.A.

INFLUENCE OF AGE AND UV ON THE POPULATIONS OF DOPA-POSITIVE MELANOCYTES IN HUMAN SKIN*

W. C. QUEVEDO, JR., PH.D., G. SZABÓ, PH.D.† AND J. VIRKS, M.D.‡

A significant decline in the numbers of 3,4-dihydroxyphenyalanine (dopa)-reactive epidermal melanocytes has been reported to occur in human skin with advancing age (1, 2). For abdominal skin, this reduction amounts to approximately a loss of 10% of the "surviving" population for each increment of 10 years (2). In contrast, a significant rise in the population of dopa-positive melanocytes within abdominal skin of females takes place during pregnancy and is accompanied by an average increase in the melanogenic activity of each melanocyte (2). A striking increase in dopa-positive melanocytes has also been found in the buttock and abdominal skin of males following repeated exposure to ultraviolet light (3, 4). These observations prompted the present study which deals with the influence of UV on the melanocyte populations of aging human skin. The site selected for examination was the skin of the buttock for reasons already reported (3). The questions to which answers have been sought are: 1) Does an age-dependent decline in the population of dopa-positive melanocytes also occur in the skin of the buttock? 2) If so, does repeated exposure to UV restore their numbers to a common value regardless of age?

MATERIALS AND METHODS

Each of twelve adult Caucasian males ranging in age from 27 to 65 years was shielded so that either the left or right buttock was exposed to UV emitted by a General Electric R.S. type sunlamp. The lamp-to-target distance was 15 inches. Between 10 and 15 treatments with erythema-producing UV were administered to each subject over approximately a two-week period. The daily doses were graded according to skin tolerance. Twenty-four hours after the last treatment, punch biopsies of skin were removed from the irradiated and contralateral shielded sites. The pairs of specimens were mounted epidermis down on coverslips to which a thin layer of silicone grease had been applied. The Staricco-Pinkus sodium bromide method was used to prepare sheets of epidermis (5). The dermis was easily separated from the epidermis after the plugs of skin had been incubated for 3 hours in an aqueous solution of 2M NaBr at 37° C. The epidermal sheets were fixed for one hour in 10% formalin (4% formaldehyde), washed and incubated in buffered dopa-solution (pH 7.4) for five hours at 37° C; the dopa-reagent was replaced after one hour (6). The epidermal sheets were then stained with Mayer's alcoholic carmine, dehydrated, cleared and prepared as whole mounts.

The numbers of melanocytes were estimated per mm^2 over the surface area of the skin in plane projection. In each specimen, the calculations were based on ten randomly chosen regions corresponding to a total area of 0.27 mm^2.

RESULTS

With the exception of an initial erythema in all cases and a slight epidermal scaling which was noted occasionally, there was no macroscopic evidence of damage to the skin during the period of treatment with UV. A striking tanning of the irradiated skin was evident in all subjects. No grossly detectable change in color or texture occurred within the shielded contralateral skin. The results of melanocyte counts are summarized in Table 1 and Figure 1. A highly significant ($P < .01$) decline in the numbers of dopa-positive melanocytes occurs in non-irradiated buttock skin with advancing age. The reduction amounts to approximately 20% of the surviving population for each increment of ten years between the ages of 27 and 65 years. Exposure to UV at all ages results in a marked increase in the numbers of dopa-reactive epidermal melanocytes. However, as in non-irradiated skin, the numbers of dopa-reactive melanocytes within irradiated skin decline with advancing age (approximately 15% of the surviving population per 10 years

Received August 16, 1968; accepted for publication September 10, 1968.

Project supported at Brown University in part by PHS Research Grant No. CA-06097-03 from the National Cancer Institute, and PHS Training Grant No. GM-00582-04 from the Division of General Medical Sciences, United States Public Health Service.

Project supported at Harvard University by USPHS Research Grant No. CA-05401 from the National Cancer Institute.

* From the Division of Biological and Medical Sciences, Brown University, Providence, R.I., the Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Mass.,† and the Rhode Island Medical Center, Howard, R.I.‡

TABLE I

Average number of melanocytes/mm² ± S.E. mean

Subject #	Age	Control	After UV radiation
1	27	1460 ± 70	2725 ± 120
2	33	1630 ± 65	3570 ± 80
3	37	1115 ± 90	2000 ± 50
4	39	950 ± 85	1715 ± 50
5	40	875 ± 60	2620 ± 75
6	42	1030 ± 90	2600 ± 90
7	46	975 ± 50	2155 ± 90
8	47	1215 ± 70	1835 ± 65
9	53	745 ± 40	1675 ± 80
10	57	760 ± 60	1970 ± 110
11	60	655 ± 55	1960 ± 80
12	65	700 ± 45	1585 ± 60

Figures rounded off to nearest five or ten melanocytes.

of life). The correlation between decline in melanocyte number and age is statistically significant ($P < .02$). Although no counts were made, the numbers of dopa-reactive melanocytes were also increased in the upper external root sheaths of hair follicles and in the upper portions of sweat gland ducts after UV. The irradiated epidermal melanocytes were usually more highly reactive with dopa than their non-irradiated counterparts and appeared to possess generally larger cell bodies and more elaborate dendrites.

DISCUSSION

The results of the present study indicate that in the skin of the buttock, as previously reported for abdominal skin (2), there is a progressive reduction in the populations of

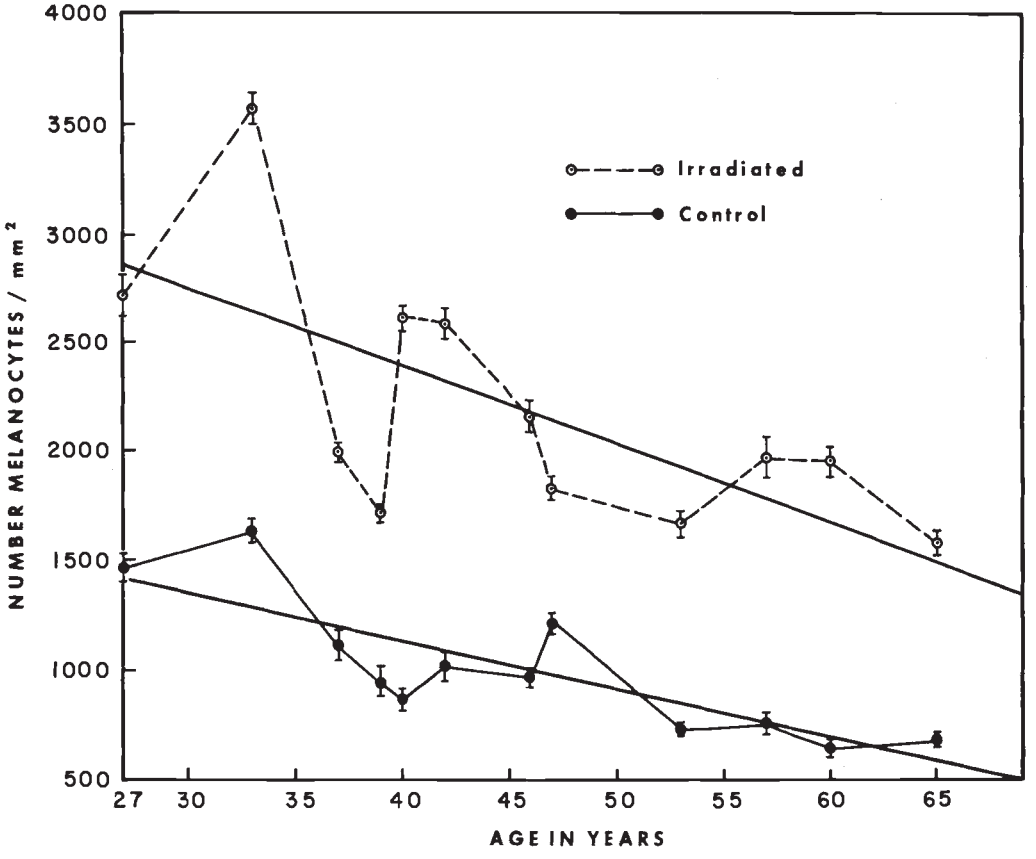

FIG. 1. Age-dependent changes in populations of dopa-reactive epidermal melanocytes. For each age sampled, the pair of points designates the average number of melanocytes/mm² ± S.E. mean in the irradiated and non-irradiated skin of the same individual. The age-dependent trends shown by melanocyte populations in irradiated and non-irradiated skin are indicated by the regression lines obtained by the method of least squares.

dopa-positive melanocytes with advancing age. This age-dependent reduction is true for melanocyte numbers after UV-radiation despite the demonstration that detectable melanocytes in all cases are increased by this treatment. The rate of decline before radiation is approximately 20% of the surviving population for each 10 years across the age range examined, and is roughly double that reported by Snell and Bischitz (2) for the abdominal skin. The mechanism accounting for the increase in dopa-reactive melanocytes within irradiated buttock skin at all ages is not clear.

Based on the great variation in dopa-reactivity and, presumably, levels of melanogenic activity within non-irradiated melanocytes, it has been suggested that UV elicits increased melanogenic activity in many melanocytes which normally produce little or no melanin (3, 7–9). The weakly melanogenic melanocytes may not darken demonstrably on incubation in dopa-reagent and thereby escape detection in sheets of non-irradiated epidermis. The uniformly intense dopa-reactivity of irradiated epidermal melanocytes gives the impression that the normally variable population has been stimulated to a comparable elevated level of melanogenic activity (3). Mishima and Widlan (9) have demonstrated by a quantitative light- and electron-histochemical study that dopa-reagent alone does not reveal all epidermal melanocytes in non-irradiated skin. They have used a combined dopa-premelanin reaction employing ammoniacal silver nitrate and gold chloride (10). In addition, they conclude that the increase in numbers of melanocytes after UV derives from increased melanogenic activity within existing melanocytes as well as from some other source possibly involving melanocyte proliferation (9). Although melanocytes have been observed to undergo division (11), the regularity with which this process occurs has not been established. The nature of the preparation of epidermal sheets in the present study did not permit an evaluation of this possibility. Quevedo *et al.* (12) have concluded, based on the occurrence of division figures within melanocytes and the incorporation of ^{3}H-thymidine within their nuclei, that melanocyte division accounts at least for part of populational increase in UV-irradiated mouse skin. No comparable studies have been made on human skin.

It is noteworthy that a maximum of 1628 dopa-positive melanocytes/mm^2 was recorded in the non-irradiated buttock skin. In all cases, UV treatment elicited populations of dopa-positive melanocytes which exceeded the maximum observed for non-irradiated skin.

Based on the comprehensive study of Mishima and Widlan (9) it now appears definite that there normally exists in the skin a pool of melanocytes with little or no dopa-reactivity and which serves as a reserve upon which UV draws in producing populational "increases". The present study suggests that irrespective of age in non-irradiated skin, there is a standing population of relatively inactive melanocytes. The age-dependent reduction in the numbers of dopa-positive irradiated melanocytes roughly parallels that found for non-irradiated melanocytes. Tentatively, this would suggest that UV induces the appearance of a relatively fixed number of new dopa-reactive melanocytes either through activation and/or proliferation or some other as yet undemonstrated mechanism. With the application of combined autoradiographic and electron microscopic techniques to this study, certain of the questions raised herein may be resolved.

SUMMARY

A group of adult Caucasian males ranging in age from 27 to 65 was exposed frequently over a two week period to UV. The treatment was restricted to the skin of the left or right buttock which tanned significantly during the period of treatment. Counts of dopa-reactive epidermal melanocytes in sheets of epidermis from the buttocks revealed: 1) in non-irradiated skin, great variation in dopa-reactivity and a decline in melanocyte numbers which is significantly correlated with advancing age, approximately 20% per 10 years of life in the age group examined; 2) more uniform dopa-reactivity in the epidermal melanocytes of irradiated skin and a significant increase in their numbers at all ages.

REFERENCES

1. Szabó, G.: Quantitative histological investigations on the melanocyte system of the human epidermis, pp. 99–125, *Pigment Cell Biology*. Ed., Gordon M. Academic Press, New York, 1959.
2. Snell, R. S. and Bischitz, P. G.: The melanocytes and melanin in human abdominal wall skin: A survey made at different ages in both

sexes and during pregnancy. J. Anat., *97:* 361, 1963.
3. Quevedo, W. C., Jr., Szabó, G. and Virks, J.: Melanocyte populations in UV-irradiated human skin. J. Invest. Derm., *45:* 295, 1965.
4. Szabó, G.: Photobiology of melanogenesis: Cytological aspects with reference to differences in racial coloration, pp. 379–396, *The Pigmentary System, Advances in Biology of Skin,* Vol. 8. Eds., Montagna, W. and Hu, F., Pergamon Press, Oxford, 1967.
5. Staricco, R. J. and Pinkus, H.: Quantitative and qualitative data on the pigment cells of adult human epidermis. J. Invest. Derm., *28:* 33, 1957.
6. Laidlaw, G. F. and Blackberg, S. N.: Melanoma studies. II. A simple technique for the dopa reaction. Amer. J. Path., *8:* 491, 1932.
7. Snell, R. S.: The effect of ultraviolet irradiation on melanogenesis. J. Invest. Derm., *40:* 127, 1963.
8. Mishima, Y. and Tanay, A.: The effect of α-methyldopa and ultraviolet irradiation on melanogenesis. Dermatologica, *136:* 105, 1968.
9. Mishima, Y. and Widlan, S.: Enzymically active and inactive melanocyte populations and ultraviolet irradiation: Combined dopa-premelanin reaction and electron microscopy. J. Invest. Derm., *49:* 273, 1967.
10. Mishima, Y.: New technique for comprehensive demonstration of melanin, premelanin, and tyrosinase sites. J. Invest. Derm., *34:* 355, 1960.
11. Giacometti, L. and Allegra, F.: The effect of wounding upon the uptake of ^{3}H-thymidine by guinea-pig epidermal melanocytes, pp. 89–95, *The Pigmentary System, Advances in Biology of Skin,* Vol. 8. Eds., Montagna, W. and Hu, F., Pergamon Press, Oxford, 1967.
12. Quevedo, W. C., Jr., Brenner, R. M. and Kechijian, P.: Melanocyte performance during radiation-induced tanning of murine skin. Proc. XVI International Congress of Zoology, *2:* 306, 1963.